Mass Spectrometric $^{13}C/^{12}C$ Determinations to Distinguish Honey and C_3 Plant Sirups From C_4 Plant Sirups (Sugar Cane and Corn) In Candied Pineapple and Papaya

LANDIS W. DONER, DAVID CHIA,[1] and JONATHAN W. WHITE, JR
U.S. Department of Agriculture, Science and Education Administration, Eastern Regional Research Center, Philadelphia, PA 19118

Stable carbon isotope ratio analysis was applied to concentrated extracts of authentic honey-processed pineapple and papaya samples and imported candied samples alleged to have been processed with honey. The 9 imported samples had $\delta^{13}C$ values ranging from −11.1 to −15.0‰, while pineapple and papaya samples known to be processed with honey had values of −25.0 and −25.2‰, respectively. The results show that the imported samples were processed with sugar sirups derived from plants using the Hatch-Slack (C_4) photosynthetic cycle, such as sugar cane and corn, rather than with honey which is derived from Calvin (C_3) floral sources.

Candied or glacé fruits are used extensively as a confection, and large quantities of sugar sirup-treated pineapple (*Ananas comosus* L., Merr.) and papaya (*Carica papaya* L.) are imported from the Orient. The import tariff levied under the Generalized System of Preferences (GSP) (1) by the U.S. Customs Service on fruits such as papaya processed with honey and/or other sugar sirups is substantially different from that applied to dehydrated papaya. The identification and verification of the ingredients used in the imported processed foods, as claimed by the importer or manufacturer, is also the responsibility of the U.S. Customs Service. Therefore, the Customs Laboratories require specific methodology to distinguish those processed with honey and/or sugar sirups from the dehydrated fruits.

Recently, a mass spectrometric determination of $^{13}C/^{12}C$ ratios was adopted as official first action for the detection of high fructose corn sirup (HFCS) in honey (2). Earlier, Bricout *et al.* used this method to detect synthetic vanillin in vanilla extracts (3). This method takes advantage of the fact that plants (and their derived products) fixing carbon dioxide via the Calvin (C_3) photosynthetic cycle, such as all honey floral sources (4, 5), have characteristically different $\delta^{13}C$ values from plants using the Hatch-Slack (C_4) cycle, such as sugar cane and corn (6). The range of $\delta^{13}C$ values for 119 imported and domestic honey samples was −22.5 to −27.4‰, while all 4 HFCS samples tested were relatively rich in ^{13}C, with $\delta^{13}C$ values ranging from −9.5 to −9.8‰. Sugar cane is also a C_4 plant, and sucrose derived from cane averages −11.1‰, with a narrow range found for several samples (7).

In this paper, we demonstrate that $\delta^{13}C$ values of sirups isolated from candied papaya and pineapple can be used to distinguish honey-processed pineapple and papaya samples from those processed with sirups from C_4 plant sources, such as corn and cane sirups. Also, papaya has been established to be a C_3 plant, and pineapple is confirmed as a Crassulacean acid metabolism (CAM) plant, capable of both C_3 and C_4 carbon dioxide fixation.

Experimental

Apparatus

Carbon isotope ratios were determined by Geochron Laboratories Division, Kreuger Enterprises, Inc., Cambridge, MA 02139, on an AEI MS-20 double collecting, 180° sector mass spectrometer with a dual capillary inlet. Further details of the $\delta^{13}C$ determinations are included elsewhere (4, 5).

Sample Preparation

Imported samples of dried processed pineapple and papaya were received at U.S. Customs Service, San Francisco, CA. Some were declared by the exporters to be processed with honey, and others with sugar sirup. Pineapple and papaya samples candied with authentic honey were prepared from the fresh fruits by a procedure similar to that described by Tressler (8). The fruits were placed in honey that had been diluted to a 30% solids level, boiled 2 min, cooled to 25°C, and then left

[1] U.S. Treasury Department, U.S. Customs Service, San Francisco, CA 94111.

24 hr. The liquid was decanted and this procedure was repeated, first with honey of 50% solids, and then with full density honey. The samples were thoroughly drained and dried at 50°C in a vacuum oven for 4 days.

Carbon isotope analysis was applied to concentrated extracts from the candied fruits by the following procedure: About 3.0 g sample was placed in a Waring blender equipped with a screw-top jar, and 25 mL 50% aqueous ethanol was added; after soaking 1 hr, the sample was homogenized for 1 min. The sides of the blender were rinsed with an additional 25 mL 50% aqueous ethanol, and the mixture was blended again 1 min. After being centrifuged at $10,000 \times g$ for 10 min, the decantate was transferred into a tared 100 mL beaker and evaporated to sirup on a steam bath under a stream of nitrogen. The residue was transferred to a 5 mL vial, in which the sirup thickened when cooled. The yield of material from both candied pineapple and papaya averaged 70%, ranging from 54 to 85% for 12 samples.

$\delta^{13}C$ determinations also were made directly on lyophilized unprocessed pineapple and papaya and on concentrated extracts prepared from the fruits as described above. Yields of fruit sirups from pineapple and papaya were 11.6 and 1.1%, respectively.

Results and Discussion

Preliminary to determining $\delta^{13}C$ values for imported pineapple and papaya samples alleged to have been processed with honey, it was necessary to analyze authentic honeyed samples, the native fruits, and concentrated extracts of the native fruits. These are reported in Table 1; the $\delta^{13}C$ values for concentrated extracts of honey candied pineapple and papaya samples are close to that of the pure alfalfa honey with which they were processed, −24.7‰. This would be expected, because candied fruits are heavily loaded with the processing sirups, as indicated from the yields obtained upon extraction. The $\delta^{13}C$ values for the lyophilized fruits establish that papaya is a Calvin (C_3) plant and confirm (9) pineapple to be a CAM plant. An unexpectedly large 5‰ depletion of ^{13}C in extracts of fresh pineapple as compared to the intact fruit was found, while the papaya extract possessed a $\delta^{13}C$ value identical to that of the intact fruit. Constituents of a given plant, however, often give a rather wide range of $\delta^{13}C$ values (10).

Table 1. $\delta^{13}C$ values of pineapple and papaya samples

Sample description	Pineapple	Papaya
Authentic honey-processed, concentrated extract	−25.0	−25.2
Lyophilized fresh fruit	−12.9	−26.2
Fresh fruit, concentrated extract	−17.9	−26.2

Table 2. $\delta^{13}C$ values of concentrated extracts from commercial candied pineapple and papaya

Sample[a]	Pineapple	Papaya
1	−15.0	−13.8
2	−14.8	−13.3
3	−12.5	
4	−11.8	
5	−11.7	
6	−11.7	
7	−11.1	
8	−16.7	

[a] Samples 1–7 were imported products; Sample 8 was purchased locally and was labeled as "organic honey-dipped pineapple."

It is clear that, regardless of the $\delta^{13}C$ value characteristic of the native fruit, the value obtained for concentrated extracts from candied fruits closely reflects the isotope ratio of the sirup used for processing, and extracts of fruits processed with sirups derived from C_4 plants, including sugar cane and corn, would have $\delta^{13}C$ values characteristic of these sirups and would be rich in ^{13}C when compared to samples processed with derivatives of C_3 plants, such as honey.

Table 2 is a compilation of ^{13}C data for imported samples of candied pineapple and papaya, 4 of which are alleged to have been processed with honey. Included is a sample (No. 8) purchased locally, labeled "organic honey-dipped pineapple." It can be concluded that Samples 3–7 (Table 2) had been processed with sugar sirups and not honey, and that some honey may have been included (or sirups from other C_3 plant sources) in the sirups used to process pineapple Samples 1, 2, and 8. The $\delta^{13}C$ values clearly indicate the predominance of C_4 plant-derived sirups. In addition, preliminary sugar analyses of the extracts showed that inverted sucrose, rather than honey, was used in the manufacturing process.

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture or the U.S. Customs Service over others of a similar nature not mentioned.

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